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(54) Title: METHODS FOR REGULATING GASTROINTESTINAL MOTILITY

(57) Abstract

Methods for reducing gastric motility and delaying gastric emptying for therapeutic and diagnostic purposes are disclosed which comprise administration of an effective amount of an exendin or an exendin agonist. Methods for treating conditions associated with elevated, inappropriate, or undesired post-prandial blood glucose levels are disclosed which comprise administration of an effective amount of an exendin or an exendin agonist alone or in conjunction with other anti-gastric emptying agents.

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METHODS FOR REGULATING GASTROINTESTINAL MOTILITYRelated Application

This application is continuation-in-part of U.S. Patent Application Serial No. 08/694,954 filed August 8, 1996, the contents of which are hereby incorporated by this reference.

Field of the Invention

The present invention relates to methods for regulating gastrointestinal motility. More particularly, the invention relates to the use of exendins and analogs and agonists thereof for the treatment of disorders which would be benefited with agents useful in delaying and/or slowing gastric emptying.

Background

The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art to the presently claimed invention, nor that any of the publications specifically or implicitly referenced are prior art to that invention.

Publications and other materials including patents and patent applications used to illuminate the specification are hereby incorporated by reference.

Exendin

The exendins are peptides that are found in the venom of the Gila-monster, a lizard found in Arizona. Exendin-3 [SEQ. ID. NO. 1] is present in the venom of

Heloderma horridum, and exendin-4 [SEQ. ID. NO. 2] is present in the venom of Heloderma suspectum (Eng, J., et al., J. Biol. Chem., 265:20259-62, 1990; Eng, J., et al., J. Biol. Chem., 267:7402-05, 1992). The exendins have
5 some sequence similarity to several members of the glucagon-like peptide family, with the highest homology, 53%, being to GLP-1[7-36]NH₂ (Goke, et al., J. Biol. Chem., 268:19650-55, 1993). GLP-1[7-36]NH₂ [SEQ. ID. NO. 3] is also known as proglucagon[78-107], or simply the
10 shorthand "GLP-1," which is used interchangeably with GLP-1[7-36]NH₂ throughout this application. The sequences of exendin-3, exendin-4 and GLP-1 are shown in Figure 1. GLP-1 has an insulinotropic effect, stimulating insulin secretion from pancreatic β -cells; GLP-1 also inhibits
15 glucagon secretion from pancreatic α -cells (Ørskov, et al., Diabetes, 42:658-61, 1993; D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). GLP-1 is reported to inhibit gastric emptying (Willms B, et al., J. Clin. Endocrinol Metab 81 (1): 327-32, 1996; Wettergren A, et
20 al., Dig Dis Sci 38 (4): 665-73, 1993), and gastric acid secretion. Schjoldager BT, et al., Dig Dis Sci 34 (5): 703-8, 1989; O'Halloran DJ, et al., J Endocrinol 126 (1): 169-73, 1990; Wettergren A, et al., Dig Dis Sci 38 (4): 665-73, 1993). GLP-1[7-37], which has an additional
25 glycine residue at its carboxy terminus, also stimulates insulin secretion in humans (Ørskov, et al., Diabetes, 42:658-61, 1993).

A transmembrane G-protein adenylate-cyclase-coupled receptor believed to be responsible for the insulinotropic
30 effect of GLP-1 has been cloned from a β -cell line (Thorens, Proc. Natl. Acad. Sci. USA 89:8641-45 (1992), hereinafter referred to as the "cloned GLP-1 receptor."

Exendin-4 is reportedly a potent agonist
receptors on insulin-secreting β TC1 cells, at diP-1
acinar cells from guinea pig pancreas, and at parD
cells from stomach; the peptide is also reported
5 stimulate somatostatin release and inhibit gastrin release
in isolated stomachs (Goke, et al., J. Biol. Chem.
268:19650-55, 1993; Schepp, et al., Eur. J. Pharmacol.,
69:183-91, 1994; Eissele, et al., Life Sci., 55:629-34,
1994). Exendin-3 and exendin-4 were found to be GLP-1
10 agonists in stimulating cAMP production in, and amylase
release from, pancreatic acinar cells (Malhotra, R., et
al., Regulatory Peptides, 41:149-56, 1992; Raufman, et al.,
J. Biol. Chem. 267:21432-37, 1992; Singh, et al., Regul.
Pept. 53:47-59, 1994). Based on the insulinotropic
15 activities of exendin-3 and exendin-4, their use has been
proposed for the treatment of diabetes mellitus and the
prevention of hyperglycemia (Eng, U.S. Patent No.
5,424,286).

In contrast to the full-length exendins, truncated
20 exendin peptides such as exendin[9-39], a carboxyamidated
molecule, and fragments 3-39 through 9-39 of exendin have
been reported to be potent and selective antagonists of
GLP-1 (Goke, et al., J. Biol. Chem., 268:19650-55, 1993;
Schepp, W., et al., Eur. J. Pharm. 269:183-91, 1994;
25 Montrose-Rafizadeh, et al., Diabetes, 45(Suppl. 2):152A,
1996). Exendin[9-39], the sequence of which is shown in
Figure 1, reportedly blocks endogenous GLP-1 in vivo,
resulting in reduced insulin secretion. Wang, et al., J.
Clin. Invest., 95:417-21, 1995; D'Alessio, et al., J.
30 Clin. Invest., 97:133-38, 1996). Exendins and
exendin[9-39] bind to the cloned GLP-1 receptor (Fehmann
HC, et al., Peptides 15 (3): 453-6, 1994; Thorens B, et

al., *Diabetes* 42 (11): 1678-82, 1993).
transfected with the cloned GLP-1 receptor, exendin¹¹
an agonist, i.e., it increases cAMP, while exendin⁹
is an antagonist, i.e., it blocks the stimulatory action
5 of exendin-4 and GLP-1.

Exendin[9-39] is also reported to act as an
antagonist of the full length exendins, inhibiting
stimulation of pancreatic acinar cells by exendin 3 and
exendin 4 (Raufman, et al., *J. Biol. Chem.* 266:2897-902,
10 1991; Raufman, et al., *J. Biol. Chem.*, 266:21432-37,
1992). Exendin[9-39] is said to inhibit the stimulation
of plasma insulin levels by exendin 4, and inhibits the
somatostatin release-stimulating and gastrin release-
inhibiting activities of exendin-4 and GLP-1 (Kolligs, F.,
15 et al., *Diabetes*, 44:16-19, 1995; Eissele, et al., *Life*
Sciences, 55:629-34, 1994).

Agents which serve to delay gastric emptying have
found a place in medicine as diagnostic aids in gastro-
intestinal radiologic examinations. For example, glucagon
20 is a polypeptide hormone which is produced by the α cells
of the pancreatic islets of Langerhans. It is a
hyperglycaemic agent which mobilizes glucose by activating
hepatic glycogenolysis. It can to a lesser extent
stimulate the secretion of pancreatic insulin. Glucagon
25 is used in the treatment of insulin-induced hypoglycaemia
when administration of glucose intravenously is not
possible. However, as glucagon reduces the motility of
the gastro-intestinal tract it is also used as a
diagnostic aid in gastro-intestinal radiological
30 examinations. Glucagon has also been used in several
studies to treat various painful gastro-intestinal
disorders associated with spasm. Daniel, et al. (*Br. Med.*

J., 1974, 3, 720) reported quicker symptomatic relief of acute diverticulitis in patients treated with glucagon compared with those who had been treated with analgesics or antispasmodics. A review by Glauser, et al., (J. Am. Coll. Emergency Physns, 8:228, 1979) described relief of acute oesophageal food obstruction following glucagon therapy. In another study glucagon significantly relieved pain and tenderness in 21 patients with biliary tract disease compared with 22 patients treated with placebo (M.J. Stower, et al., Br. J. Surg., 69:591-2, 1982).

Methods for regulating gastrointestinal motility using amylin agonists are described in International Application No. PCT/US94/10225, published March 16, 1995.

SUMMARY OF THE INVENTION

The present invention concerns the surprising discovery that exendins are potent inhibitors of gastric emptying. Exendins and exendin agonists are useful as inhibitors of gastric emptying for the treatment of, for example, diabetes mellitus, obesity, the ingestion of toxins, or for diagnostic purposes.

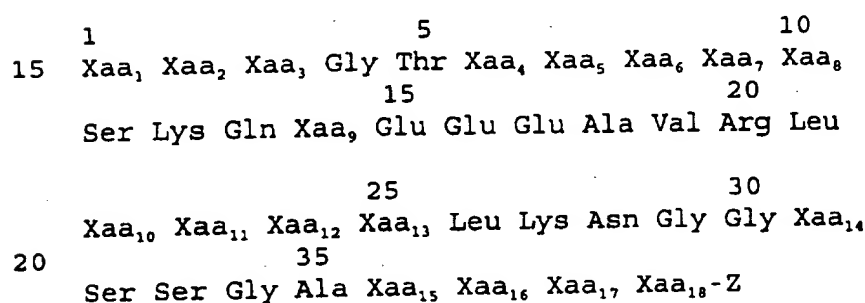
The present invention is directed to novel methods for reducing gastric motility and slowing gastric emptying, comprising the administration of an exendin, for example, exendin 3 [SEQ ID NO. 1], exendin 4 [SEQ ID NO. 2], or other compounds which effectively bind to the receptor at which exendins exert their action on gastric motility and gastric emptying. These methods will be useful in the treatment of, for example, post-prandial hyperglycemia, a complication associated with type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes mellitus.

In a first aspect, the invention features a method of beneficially regulating gastrointestinal motility in a subject by administering to said subject a therapeutically effective amount of an exendin or an exendin agonist. By

5 "exendin agonist" is meant a compound which mimics the effects of exendins on gastric motility and gastric emptying, namely, a compound which effectively binds to the receptor at which exendins exert their action on gastric motility and gastric emptying, preferably an

10 analog or derivative of an exendin.

Exendin agonist compounds useful in present invention include those compounds of the formula (I) [SEQ. ID. NO. 4]:



wherein Xaa₁ is His, Arg or Tyr; Xaa₂ is Ser, Gly, Ala or Thr; Xaa₃ is Asp or Glu; Xaa₄ is Phe, Tyr or naphthalanine; Xaa₅ is Thr or Ser; Xaa₆ is Ser or Thr; Xaa₇ is Asp or Glu; Xaa₈ is Leu, Ile, Val, pentylglycine or Met; Xaa₉ is Leu, Ile, pentylglycine, Val or Met; Xaa₁₀ is Phe, Tyr or naphthalanine; Xaa₁₁ is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met; Xaa₁₂ is Glu or Asp; Xaa₁₃ is Trp, Phe, Tyr, or naphthylalanine; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine; Xaa₁₈ is Ser, Thr or Tyr; and Z is -OH or -NH₂; with the proviso that the

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compound does not have the formula of either SEQ. ID. NOS. 1 or 2. Also useful in the present invention are pharmaceutically acceptable salts of the compounds of formula (I).

5 In one embodiment, the methods of the present invention are directed to reducing gastric motility. In another embodiment, the invention is directed to methods of delaying gastric emptying.

These methods may be used on a subject undergoing a
10 gastrointestinal diagnostic procedure, for example radiological examination or magnetic resonance imaging. Alternatively, these methods may be used to reduce gastric motility in a subject suffering from a gastro-intestinal disorder, for example, spasm (which may be associated with
15 acute diverticulitis, a disorder of the biliary tract or a disorder of the Sphincter of Oddi).

In another aspect, the invention is directed to a method of treating post-prandial dumping syndrome in a subject by administering to the subject a therapeutically
20 effective amount of an exendin or exendin agonist.

In yet another aspect, the invention is directed to a method of treating post-prandial hyperglycemia by administering to a subject a therapeutically effective amount of an exendin or exendin agonist. In a preferred
25 embodiment, the post-prandial hyperglycemia is a consequence of Type 2 diabetes mellitus. In other preferred embodiments, the post-prandial hyperglycemia is a consequence of Type 1 diabetes mellitus or impaired glucose tolerance.

30 In another aspect, a therapeutically effective amount of an amylin agonist is also administered to the subject. In a preferred aspect, the amylin agonist is an amylin or

an amylin agonist analog such as ^{25,28,29}Pro-human-amylin. The use of amylin agonists to treat post-prandial hyperglycemia, as well as to beneficially regulate gastrointestinal motility, is described in International
5 Application No. PCT/US94/10225, published March 16, 1995 which has been incorporated by reference herein.

In yet another aspect, a therapeutically effective amount of an insulin or insulin analog is also administered, separately or together with an exendin or
10 exendin agonist, to the subject.

In another aspect, the invention is directed to a method of treating ingestion of a toxin by administering an amount of an exendin or an exendin agonist effective to prevent or reduce passage of stomach contents to the
15 intestines and aspirating the stomach contents.

Definitions

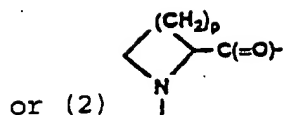
In accordance with the present invention and as used herein, the following terms are defined to have the following meanings, unless explicitly stated otherwise.

20 The term "amino acid" refers to natural amino acids, unnatural amino acids, and amino acid analogs, all in their D and L stereoisomers if their structure allow such stereoisomeric forms. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid
25 (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), Lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), typtophan (Trp), tyrosine (Tyr) and
30 valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid,

3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, tertiary-
5 butylglycine, 2,4-diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, homoproline, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-
10 methylalanine, N-methylglycine, N-methylisoleucine, N-methylpentylglycine, N-methylvaline, naphthalanine, norvaline, norleucine, ornithine, pentylglycine, pipercolic acid and thioproline. Amino acid analogs include the natural and unnatural amino acids which are chemically
15 blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone.

20 The term "amino acid analog" refers to an amino acid wherein either the C-terminal carboxy group, the N-terminal amino group or side-chain functional group has been chemically codified to another functional group. For example, aspartic acid-(beta-methyl ester) is an amino
25 acid analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine.

The term "amino acid residue" refers to radicals having the structure: (1) $-C(O)-R-NH-$, wherein R typically
30 is $-CH(R')$, wherein R' is an amino acid side chain, typically H or a carbon containing substituent;



, wherein p is 1, 2 or 3 representing the azetidinecarboxylic acid, proline or pipercolic acid residues, respectively.

The term "lower" referred to herein in connection with organic radicals such as alkyl groups defines such groups with up to and including about 6, preferably up to and including 4 and advantageously one or two carbon atoms. Such groups may be straight chain or branched chain.

"Pharmaceutically acceptable salt" includes salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid. In practice the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention.

In addition, the following abbreviations stand for the following:

- "ACN" or "CH₃CN" refers to acetonitrile.
- "Boc", "tBoc" or "Tboc" refers to t-butoxy carbonyl.
- "DCC" refers to N,N'-dicyclohexylcarbodiimide.
- "Fmoc" refers to fluorenylmethoxycarbonyl.
- "HBTU" refers to 2-(1H-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate.
- "HOBt" refers to 1-hydroxybenzotriazole monohydrate.
- "homoP" or hPro" refers to homoproline.
- "MeAla" or "Nme" refers to N-methylalanine.
- "naph" refers to naphthylalanine.
- "pG" or pGl"y" refers to pentylglycine.
- "tBuG" refers to tertiary-butylglycine.
- "ThioP" or tPro" refers to thioproline.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a comparison of the amino acid sequences of exendin 3, exendin 4, and exendin[9-39] using standard single letter rather than three letter amino acid codes.

FIGURE 2 shows GLP-1[7-36]NH₂, exendin-3 and exendin-4 dose-response effects of prior subcutaneous injection on the retention of gastric contents 20 minutes after gavage in normal rats (n = 3-17 for each point). Symbols are means + SEM and the curves define the best fitting logistic functions. "Zero" indicates the fraction of gastric contents retained in untreated normal rats.

FIGURE 3 shows the dose response effects of prior injection of exendin-4 (n = 29), exendin-4 acid (n = 36) and ¹⁴Leu, ²⁵Phe exendin-4 (n = 36) on the retention of gastric contents 20 minutes after gavage in normal rats. Symbols are means plus or minus standard error of the mean and the curves define the best fitting logistic functions. "Zero" indicates the fraction of gastric contents retained in untreated normal rats.

FIGURE 4 shows the effect of prior injection of 1.0 µg exendin-4 (sc), n=6; 1.0 µg exendin-4 (sc) plus 0.3 mg exendin[9-39] (sc), n=6; and 0.3 mg exendin[9-39] (sc), n=6 on the retention of gastric contents 20 minutes after gavage. Also shown are saline controls at t=0 and t=20 min. The error bars show standard error of the mean. As shown in FIGURE 4, exendin-4 alone potently inhibited gastric emptying. Exendin[9-39] (sc) alone had no effect on gastric emptying. When injected along with exendin-4, exendin[9-39] did not antagonize the effect of exendin-4 on gastric emptying inhibition.

FIGURE 5 shows the effect of prior injection of 0.3 μ g exendin-4 (sc), n=5 and 0.3 μ g exendin-4 (sc) plus 0.5 mg exendin[9-39] (iv), n=5 on the retention of gastric contents 20 minutes after gavage. Also shown are saline controls at t=0 and t=20 min. The error bars show standard error of the mean. As shown in FIGURE 5, exendin-4 alone potentially inhibited gastric emptying. When injected along with exendin-4, exendin[9-39] (iv) did not antagonize the effect of exendin-4 on gastric emptying inhibition.

FIGURE 6 shows the effect of prior injection of 10 μ g GLP-1[7-36]NH₂ (sc), n=8; 10 μ g GLP-1[7-36]NH₂ (sc) plus 3 mg exendin[9-39] (sc), n=6; and 0.3 mg exendin[9-39] (sc), n=6 on the retention of gastric contents 20 minutes after gavage. Also shown are saline controls at t=0 and t=20 min. The error bars show standard error of the mean. As shown in FIGURE 6, GLP-1[7-36]NH₂ potentially inhibited gastric emptying. Exendin[9-39] (sc) alone had no effect on gastric emptying. When injected along with GLP-1[7-36]NH₂, exendin[9-39] did not antagonize the effect of GLP-1[7-36]NH₂ on gastric emptying inhibition.

FIGURE 7 shows the effect of prior injection of 10 μ g GLP-1[7-36]NH₂ (sc), n=8, and 10 μ g GLP-1[7-36]NH₂ (sc) plus 0.5 mg exendin[9-39] (iv), n=3 on the retention of gastric contents 20 minutes after gavage. Also shown are saline controls at t=0 and t=20 min. The error bars show standard error of the mean. As shown in FIGURE 7, GLP-1[7-36]NH₂ alone potentially inhibited gastric emptying. When injected along with GLP-1[7-36]NH₂, exendin[9-39] (iv) did not antagonize the effect of GLP-1[7-36]NH₂ on gastric emptying inhibition.

FIGURE 8-1 and 8-2 depicts the amino acid sequences for certain exendin agonists [SEQ. ID. NOS. 5 TO 35].

DETAILED DESCRIPTION OF THE INVENTION

Exendins and exendin agonists (including exendin
5 analogs and exendin derivatives) are useful in this
invention in view of their pharmacological properties.
Activity as exendin agonists can be indicated by activity
in the assays described below. Effects of exendins or
exendin agonists on gastric motility and gastric emptying
10 can be identified, evaluated, or screened for, using the
methods described in Examples 1-3 below, or other art-
known or equivalent methods for determining gastric
motility. Negative receptor assays or screens for exendin
agonist compounds or candidate exendin agonist compounds,
15 such as a GLP-1 receptor preparation, an amylin receptor
assay/screen using an amylin receptor preparation as
described in U.S. Patent No. 5,264,372, issued November
23, 1993, the contents of which are incorporated herein by
reference, one or more calcitonin receptor assays/screens
20 using, for example, T47D and MCF7 breast carcinoma cells,
which contain calcium receptors coupled to the stimulation
of adenylyl cyclase activity, and/or a CGRP receptor
assay/screen using, for example, SK-N-MC cells, can be
used to evaluate and/or confirm exendin agonist activity.
25 One such method for use in identifying or evaluating
the ability of a compound to slow gastric motility,
comprises: (a) bringing together a test sample and a test
system, said test sample comprising one or more test
compounds, said test system comprising a system for
30 evaluating gastric motility, said system being
characterized in that it exhibits, for example, elevated

plasma glucose in response to the introduction to said system of glucose or a meal; and, (b) determining the presence or amount of a rise in plasma glucose in said system. Positive and/or negative controls may be used as well.

Exendins and exendin agonist compounds such as exendin analogs and exendin derivatives, described herein may be prepared through peptide purification as described in, for example, Eng, et al., J. Biol. Chem. 265:20259-62, 10 1990; and Eng, et al., J. Biol. Chem. 267:7402-05, 1992, hereby incorporated by reference herein. Alternatively, exendins and exendin agonist peptides may be prepared by methods known to those skilled in the art, for example, as described in Raufman, et al. (J. Biol. Chem. 267:21432-37, 15 1992), hereby incorporated by reference herein, using standard solid-phase peptide synthesis techniques and preferably an automated or semiautomated peptide synthesizer. Typically, an α -N-carbamoyl protected amino acid and an amino acid attached to the growing peptide 20 chain on a resin are coupled at room temperature in an inert solvent such as dimethylformamide, N-methylpyrrolidinone or methylene chloride in the presence of coupling agents such as dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in the 25 presence of a base such as diisopropylethylamine. The α -N-carbamoyl protecting group is removed from the resulting peptide-resin using a reagent such as trifluoroacetic acid or piperidine, and the coupling reaction repeated with the next desired N-protected amino 30 acid to be added to the peptide chain. Suitable N-protecting groups are well known in the art, with t-butyloxycarbonyl (tBoc) and fluorenylmethoxycarbonyl

(Fmoc) being preferred herein.

The solvents, amino acid derivatives and 4-methylbenzhydryl-amine resin used in the peptide synthesizer may be purchased from Applied Biosystems Inc. (Foster City, CA). The side-chain protected amino acids, such as Boc-Arg(Mts), Fmoc-Arg(Pmc), Boc-Thr(Bzl), Fmoc-Thr(t-Bu), Boc-Ser(Bzl), Fmoc-Ser(t-Bu), Boc-Tyr(BrZ), Fmoc-Tyr(t-Bu), Boc-Lys(Cl-Z), Fmoc-Lys(Boc), Boc-Glu(Bzl), Fmoc-Glu(t-Bu), Fmoc-His(Trt), Fmoc-Asn(Trt), and Fmoc-Gln(Trt) may be purchased from Applied Biosystems, Inc. Boc-His(BOM) may be purchased from Applied Biosystems, Inc. or Bachem Inc. (Torrance, CA). Anisole, methylsulfide, phenol, ethanedithiol, and thioanisole may be obtained from Aldrich Chemical Company (Milwaukee, WI). Air Products and Chemicals (Allentown, PA) supplies HF. Ethyl ether, acetic acid and methanol may be purchased from Fisher Scientific (Pittsburgh, PA).

Solid phase peptide synthesis may be carried out with an automatic peptide synthesizer (Model 430A, Applied Biosystems Inc., Foster City, CA) using the NMP/HOBT (Option 1) system and tBoc or Fmoc chemistry (see, Applied Biosystems User's Manual for the ABI 430A Peptide Synthesizer, Version 1.3B July 1, 1988, section 6, pp. 49-70, Applied Biosystems, Inc., Foster City, CA) with capping. Boc-peptide-resins may be cleaved with HF (-5°C to 0°C, 1 hour). The peptide may be extracted from the resin with alternating water and acetic acid, and the filtrates lyophilized. The Fmoc-peptide resins may be cleaved according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc., 1990, pp. 6-12). Peptides may be also assembled using an Advanced

Chem Tech Synthesizer (Model MPS 350, Louisville, Kentucky). Peptides may be purified by RP-HPLC (preparative and analytical) using a Waters Delta Prep 3000 system. A C4, C8 or C18 preparative column (10 μ , 2.2 x 25 cm; Vydac, Hesperia, CA) may be used to isolate peptides, and purity may be determined using a C4, C8 or C18 analytical column (5 μ , 0.46 x 25 cm; Vydac). Solvents (A=0.1% TFA/water and B=0.1% TFA/CH₃CN) may be delivered to the analytical column at a flowrate of 1.0 ml/min and to the preparative column at 15 ml/min. Amino acid analyses may be performed on the Waters Pico Tag system and processed using the Maxima program. The peptides may be hydrolyzed by vapor-phase acid hydrolysis (115°C, 20-24 h). Hydrolysates may be derivatized and analyzed by standard methods (Cohen, S.A., Meys, M., and Tarrin, T.L. (1989), The Pico Tag Method: A Manual of Advanced Techniques for Amino Acid Analysis, pp. 11-52, Millipore Corporation, Milford, MA). Fast atom bombardment analysis may be carried out by M-Scan, Incorporated (West Chester, PA). Mass calibration may be performed using cesium iodide or cesium iodide/glycerol. Plasma desorption ionization analysis using time of flight detection may be carried out on an Applied Biosystems Bio-Ion 20 mass spectrometer.

Peptide compounds useful in the invention may also be prepared using recombinant DNA techniques, using methods now known in the art. See, e.g., Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor (1989). Alternatively, such compounds may be prepared by homogeneous phase peptide synthesis methods.

The use of exendin analogs or derivatives is included within the methods of the present invention. Exendin analogs or derivatives are functional variants having similar amino acid sequence and retaining, to some extent, at least the gastric motility- and gastric emptying-related activities of the related exendin. By "functional variant" is meant an analog or derivative which has an activity that can be substituted for one or more activities of a particular exendin. Preferred functional variants retain all of the activities of a particular exendin, however, the functional variant may have an activity that, when measured quantitatively, is stronger or weaker, as measured in exendin functional assays, for example, such as those disclosed herein. Preferred functional variants have activities that are within about 1% to about 10,000% of the activity of the related exendin, more preferably between about 10% to about 1000%, and more preferably within about 50% to about 500%. Derivatives have at least about 15% sequence similarity, preferably about 70%, more preferably about 90%, and even more preferably about 95% sequence similarity to the related exendin. "Sequence similarity" refers to "homology" observed between amino acid sequences in two different polypeptides, irrespective of polypeptide origin.

The ability of the analog or derivative to retain some activity can be measured using techniques described herein.

Derivatives include modification occurring during or after translation, for example, by phosphorylation, glycosylation, crosslinking, acylation, proteolytic cleavage, linkage to an antibody molecule, membrane

molecule or other ligand (see Ferguson et al., Annu. Rev. Biochem. 57:285-320, 1988).

Specific types of analogs include amino acid alterations such as deletions, substitutions, additions, and amino acid modifications. A "deletion" refers to the absence of one or more amino acid residue(s) in the related polypeptide. An "addition" refers to the presence of one or more amino acid residue(s) in the related polypeptide. Additions and deletions to a polypeptide may be at the amino terminus, the carboxy terminus, and/or internal. Amino acid "modification" refers to the alteration of a naturally occurring amino acid to produce a non-naturally occurring amino acid. A "substitution" refers to the replacement of one or more amino acid residue(s) by another amino acid residue(s) in the polypeptide. Analogs can contain different combinations of alterations including more than one alteration and different types of alterations.

Preferred analogs have one or more amino acid alteration(s) which do not significantly affect exendin agonist activity. In regions of the exendin not necessary for exendin agonist activity, amino acids may be deleted, added or substituted with less risk of affecting activity. In regions required for exendin agonist activity, amino acid alterations are less preferred as there is a greater risk of affecting exendin activity. Such alterations should be conservative alterations. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional variant.

Conserved regions tend to be more important for protein activity than non-conserved regions. Known

procedures may be used to determine the conserved and non-conserved regions important of receptor activity using in vitro mutagenesis techniques or deletion analyses and measuring receptor activity as described by the present disclosure.

Modifications to a specific polypeptide may be deliberate, as through site-directed mutagenesis and amino acid substitution during solid-phase synthesis, or may be accidental such as through mutations in hosts or systems which produce the polypeptide.

Compounds particularly useful according to the present invention are exendin agonist compounds of the formula (I) [SEQ. ID. NO. 4]:

	1		5		10
15	Xaa ₁	Xaa ₂	Xaa ₃	Gly Thr Xaa ₄	Xaa ₅ Xaa ₆ Xaa ₇ Xaa ₈
			15		20
	Ser	Lys	Gln Xaa ₉	Glu Glu Glu Ala Val Arg	Leu
			25		30
	Xaa ₁₀	Xaa ₁₁	Xaa ₁₂ Xaa ₁₃	Leu Lys Asn Gly Gly	Xaa ₁₄
20			35		
	Ser	Ser	Gly Ala Xaa ₁₅	Xaa ₁₆ Xaa ₁₇	Xaa ₁₈ -Z

wherein Xaa₁ is His, Arg or Tyr; Xaa₂ is Ser, Gly, Ala or Thr; Xaa₃ is Asp or Glu; Xaa₄ is Phe, Tyr or naphthalanine; Xaa₅ is Thr or Ser; Xaa₆ is Ser or Thr; Xaa₇ is Asp or Glu; Xaa₈ is Leu, Ile, Val, pentylglycine or Met; Xaa₉ is Leu, Ile, pentylglycine, Val or Met; Xaa₁₀ is Phe, Tyr or naphthalanine; Xaa₁₁ is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met; Xaa₁₂ is Glu or Asp; Xaa₁₃ is Trp, Phe, Tyr, or naphthylalanine; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine; Xaa₁₈ is Ser, Thr or Tyr; and Z is -OH or -NH₂; with the proviso that the

compound does not have the formula of either SEQ. ID. NOS. 1 or 2. Preferred N-alkyl groups for N-alkylglycine, N-alkylpentylglycine and N-alkylalanine include lower alkyl groups preferably of 1 to about 6 carbon atoms, more preferably of 1 to 4 carbon atoms. Suitable compounds include those having amino acid sequences of SEQ. ID. NOS. 5 to 35.

Preferred exendin agonist compounds include those wherein Xaa₁ is His or Tyr. More preferably Xaa₁ is His.

10 Preferred are those compounds wherein Xaa₂ is Gly.

Preferred are those compounds wherein Xaa₃ is Leu, pentylglycine or Met.

Preferred compounds include those wherein Xaa₁₁ is Trp or Phe.

15 Also preferred are compounds where Xaa₄ is Phe or naphthalanine; Xaa₁₁ is Ile or Val and Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently selected from Pro, homoproline, thioproline or N-alkylalanine. Preferably N-alkylalanine has a N-alkyl group of 1 to about 6 carbon atoms.

20 According to an especially preferred aspect, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are the same amino acid residue.

Preferred are compounds wherein Xaa₁₈ is Ser or Tyr, more preferably Ser.

Preferably Z is -NH₂.

25 According to one aspect, preferred are compounds of formula (I) wherein Xaa₁ is His or Tyr, more preferably His; Xaa₂ is Gly; Xaa₄ is Phe or naphthalanine; Xaa₃ is Leu, pentylglycine or Met; Xaa₁₀ is Phe or naphthalanine; Xaa₁₁ is Ile or Val; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently
30 selected from Pro, homoproline, thioproline or N-alkylalanine; and Xaa₁₈ is Ser or Tyr, more preferably Ser. More preferably Z is -NH₂.

According to an especially preferred aspect, especially preferred compounds include those of formula (I) wherein: Xaa₁ is His or Arg; Xaa₂ is Gly; Xaa₃ is Asp or Glu; Xaa₄ is Phe or naphthylalanine; Xaa₅ is Thr or Ser;
5 Xaa₆ is Ser or Thr; Xaa₇ is Asp or Glu; Xaa₈ is Leu or pentylglycine; Xaa₉ is Leu or pentylglycine; Xaa₁₀ is Phe or naphthylalanine; Xaa₁₁ is Ile, Val or t-butylglycine; Xaa₁₂ is Glu or Asp; Xaa₁₃ is Trp or Phe; Xaa₁₄, Xaa₁₅, Xaa₁₆, and Xaa₁₇ are independently Pro,
10 homoproline, thioproline, or N-methylalanine; Xaa₁₈ is Ser or Tyr; and Z is -OH or -NH₂; with the proviso that the compound does not have the formula of either SEQ. ID. NOS. 1 or 2. More preferably Z is -NH₂. Especially preferred compounds include those having the amino acid sequence of
15 SEQ. ID. NOS. 5, 6, 17, 18, 19, 22, 24, 31, 32 and 35.

According to an especially preferred aspect, provided are compounds where Xaa₈ is Leu, Ile, Val or pentylglycine, more preferably Leu or pentylglycine, and Xaa₁₃ is Phe, Tyr or naphthylalanine, more preferably Phe or
20 naphthylalanine. These compounds are believed to exhibit advantageous duration of action and to be less subject to oxidative degradation, both *in vitro* and *in vivo*, as well as during synthesis of the compound.

The compounds referenced above form salts with
25 various inorganic and organic acids and bases. Such salts include salts prepared with organic and inorganic acids, for example, HCl, HBr, H₂SO₄, H₃PO₄, trifluoroacetic acid, acetic acid, formic acid, methanesulfonic acid, toluenesulfonic acid, maleic acid, fumaric acid and
30 camphorsulfonic acid. Salts prepared with bases include ammonium salts, alkali metal salts, e.g. sodium and potassium salts, and alkali earth salts, e.g. calcium and

magnesium salts. Acetate, hydrochloride, and trifluoroacetate salts are preferred. The salts may be formed by conventional means, as by reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

10 The compounds referenced above form salts with various inorganic and organic acids and bases. Such salts include salts prepared with organic and inorganic acids, for example, HCl, HBr, H₂SO₄, H₃PO₄, trifluoroacetic acid, acetic acid, formic acid, methanesulfonic acid, 15 toluenesulfonic acid, maleic acid, fumaric acid and camphorsulfonic acid. Salts prepared with bases include ammonium salts, alkali metal salts, e.g. sodium and potassium salts, and alkali earth salts, e.g. calcium and magnesium salts. Acetate, hydrochloride, and 20 trifluoroacetate salts are preferred. The salts may be formed by conventional means, as by reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water 25 which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

The compounds described above are useful in view of their pharmacological properties. In particular, the 30 compounds of the invention possess activity as agents to regulate gastric motility and to slow gastric emptying, as evidenced by the ability to inhibit gastric emptying

levels in mammals.

As described in Example 1, gastric emptying was measured in normal Sprague Dawley rats using the retention of an acacloric methylcellulose gel containing Phenol Red delivered by gavage. Dye content in stomachs removed after sacrifice 20 minutes later was determined spectroscopically, and was compared to that in rats sacrificed immediately after gavage to assess emptying. The exendins, exendin 3 and exendin 4, dose-dependently inhibited gastric emptying. The ED_{50} of the response to exendin 3 and exendin 4 was 0.1 and 0.08 μ g, respectively, demonstrating that the exendins were -170-290 times more potent than GLP-1[7-36] NH_2 in inhibiting gastric emptying.

As described in Example 2, the effects of exendin-4 and the exendin-4 analogs, exendin-4 acid and $^{14}Leu,^{25}Phe$ exendin-4, on inhibition of gastric emptying were examined. Exendin-4 and the exendin-4 analogs dose dependently inhibiting gastric emptying. The ED_{50} of exendin-4 was 0.27 μ g. The ED_{50} s of exendin-4 acid and $^{14}Leu,^{25}Phe$ exendin-4 were 0.12 μ g and 0.29 μ g, respectively, indicating that the potency of the analogs was comparable to that of exendin-4.

As described in Example 3, the effects of exendin-4 and the cloned GLP-1 receptor antagonist, exendin[9-39] on gastric emptying were examined. After 20 minutes, the animals treated with exendin-4 showed potent inhibition of gastric emptying, which was not reversed by exendin[9-39]. This occurred regardless of whether the exendin[9-39] was administered sc or iv. Exendin[9-39] alone had no effect on gastric emptying.

As noted above, exendin[9-39] is a potent antagonist of GLP-1 which binds at the cloned GLP-1

receptor (Fehmann HC, et al., *Peptides* 15(3): 453-6, 1994; Thorens B, et al., *Diabetes* 42(11): 1678-82, 1993). Surprisingly, however, exendin[9-39] did not block the effect of exendin-4 on gastric emptying (see Figures 4 and 5). These results indicate that the effects of exendins and exendin agonists on gastric emptying are not due binding of the exendins at the cloned GLP-1 receptor, but instead that the gastric emptying effects of exendins and exendin agonists are due to their action on a separate receptor.

That exendins can act via mechanisms other than those attributable to the cloned GLP-1 receptor is further evidenced by the reported absence of effect of exendin-4 on inhibition of pentagastrin-induced gastric acid secretion, despite the inhibitory effect of GLP-1 on such secretion. Gedulin, et al., *Diabetologia*, 40(Suppl. 1):A300 (Abstract 1181) (1997). Additionally, as described in commonly assigned U.S. Provisional Patent Application Serial No. 60/034,905, entitled, "Use of Exendins and Agonists Therefor for the Reduction of Food Intake," filed January 7, 1997, peripherally injected exendin inhibited food intake in mice, an action not observed with GLP-1.

Compositions useful in the invention may conveniently be provided in the form of formulations suitable for parenteral (including intravenous, intramuscular and subcutaneous) or nasal or oral administration. In some cases, it will be convenient to provide an exendin or exendin agonist and another anti-emptying agent, such as glucagon, or amylin, or an amylin agonist, in a single composition or solution for administration together. In other cases, it may be more advantageous to administer

another anti-emptying agent separately from said exendin or exendin agonist. A suitable administration format may best be determined by a medical practitioner for each patient individually. Suitable pharmaceutically acceptable carriers and their formulation are described in standard formulation treatises, e.g., Remington's Pharmaceutical Sciences by E.W. Martin. See also Wang, Y.J. and Hanson, M.A. "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988).

Compounds useful in the invention can be provided as parenteral compositions for injection or infusion. They can, for example, be suspended in an inert oil, suitably a vegetable oil such as sesame, peanut, olive oil, or other acceptable carrier. Preferably, they are suspended in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 5.6 to 7.4. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH buffering agents. Useful buffers include for example, sodium acetate/acetic acid buffers. A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or delivery.

The desired isotonicity may be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate,

propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

The claimed compositions can also be formulated as
5 pharmaceutically acceptable salts (e.g., acid addition salts) and/or complexes thereof. Pharmaceutically acceptable salts are non-toxic salts at the concentration at which they are administered. The preparation of such salts can facilitate the pharmacological use by altering
10 the physical-chemical characteristics of the composition without preventing the composition from exerting its physiological effect. Examples of useful alterations in physical properties include lowering the melting point to facilitate transmucosal administration and increasing the
15 solubility to facilitate the administration of higher concentrations of the drug.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, phosphate, sulfamate, acetate, citrate,
20 lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric
25 acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid. Such salts may be prepared by, for example, reacting the free acid or base forms of the product with
30 one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or

by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

Carriers or excipients can also be used to facilitate administration of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars such as lactose, glucose, or sucrose, or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents. The compositions or pharmaceutical composition can be administered by different routes including intravenously, intraperitoneal, subcutaneous, and intramuscular, orally, topically, or transmucosally.

If desired, solutions of the above compositions may be thickened with a thickening agent such as methyl cellulose. They may be prepared in emulsified form, either water in oil or oil in water. Any of a wide variety of pharmaceutically acceptable emulsifying agents may be employed including, for example, acacia powder, a non-ionic surfactant (such as a Tween), or an ionic surfactant (such as alkali polyether alcohol sulfates or sulfonates, e.g., a Triton).

Compositions useful in the invention are prepared by mixing the ingredients following generally accepted procedures. For example, the selected components may be simply mixed in a blender or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control tonicity.

For use by the physician, the compositions will be provided in dosage unit form containing an amount of an exendin or exendin agonist, for example, exendin 3,

exendin 4, with or without another antiemptying agent. Therapeutically effective amounts of an exendin or exendin agonist for use in the control of gastric emptying and in conditions in which gastric emptying is beneficially
5 slowed or regulated are those that decrease post-prandial blood glucose levels, preferably to no more than about 8 or 9 mM or such that blood glucose levels are reduced as desired. In diabetic or glucose intolerant individuals, plasma glucose levels are higher than in normal
10 individuals. In such individuals, beneficial reduction or "smoothing" of post-prandial blood glucose levels, may be obtained. As will be recognized by those in the field, an effective amount of therapeutic agent will vary with many factors including the age and weight of the patient, the
15 patient's physical condition, the blood sugar level or level of inhibition of gastric emptying to be obtained, and other factors.

Such pharmaceutical compositions are useful in causing gastric hypomotility in a subject and may be used
20 as well in other disorders where gastric motility is beneficially reduced.

The effective daily anti-emptying dose of the compounds will typically be in the range of 0.001 or 0.003 to about 5 mg/day, preferably about 0.001 or 0.05 to 2
25 mg/day and more preferably about 0.001 or 0.01 to 1 mg/day, for a 70 kg patient, administered in a single or divided doses. The exact dose to be administered is determined by the attending clinician and is dependent upon where the particular compound lies within the above
30 quoted range, as well as upon the age, weight and condition of the individual. Administration should begin at the first sign of symptoms or shortly after diagnosis

of diabetes mellitus. Administration may be by injection, preferably subcutaneous or intramuscular. Orally active compounds may be taken orally, however dosages should be increased 5-10 fold.

5 Generally, in treating or preventing elevated, inappropriate, or undesired post-prandial blood glucose levels, the compounds of this invention may be administered to patients in need of such treatment in a dosage ranges similar to those given above, however, the
10 compounds are administered more frequently, for example, one, two, or three times a day.

 The optimal formulation and mode of administration of compounds of the present application to a patient depend on factors known in the art such as the particular disease
15 or disorder, the desired effect, and the type of patient. While the compounds will typically be used to treat human patients, they may also be used to treat similar or identical diseases in other vertebrates such as other primates, farm animals such as swine, cattle and poultry,
20 and sports animals and pets such as horses, dogs and cats.

 To assist in understanding the present invention, the following Examples are included. The experiments relating to this invention should not, of course, be construed as specifically limiting the invention and such variations of
25 the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

EXAMPLE 1

30 The following study was carried out to examine the effects of exendin-3 and exendin-4 on gastric emptying and

to compare the effects with GLP-1[7-36]NH₂ treatment in rats. This experiment followed a modification of the method of Scarpignato, et al., Arch. Int. Pharmacodyn. Ther. 246:286-94 (1980).

5 Male Harlan Sprague Dawley (HSD) rats were used. All animals were housed at 22.7 ± 0.8 C in a 12:12 hour light:dark cycle (experiments being performed during the light cycle) and were fed and watered ad libitum (Diet LM-485, Teklad, Madison, WI). Exendin-3 and exendin-4
10 were synthesized according to standard peptide synthesis methods.

The determination of gastric emptying by the method described below was performed after a fast of -20 hours to ensure that the stomach contained no chyme that would
15 interfere with spectrophotometric absorbance measurements.

Conscious rats received by gavage, 1.5ml of an aca-
loric gel containing 1.5% methyl cellulose (M-0262, Sigma Chemical Co, St Louis, MO) and 0.05% phenol red indicator. Twenty minutes after gavage, rats were
20 anesthetized using 5% halothane, the stomach exposed and clamped at the pyloric and lower esophageal sphincters using artery forceps, removed and opened into an alkaline solution which was made up to a fixed volume. Stomach content was derived from the intensity of the phenol red
25 in the alkaline solution, measured by absorbance at a wavelength of 560 nm. In separate experiments on 7 rats, the stomach and small intestine were both excised and opened into an alkaline solution. The quantity of phenol red that could be recovered from the upper
30 gastrointestinal tract within 20 minutes of gavage was $89 \pm 4\%$; dye which appeared to bind irrecoverably to the gut luminal surface may have accounted for the balance. To

account for a maximal dye recovery of less than 100%, percent of stomach contents remaining after 20 min were expressed as a fraction of the gastric contents recovered from control rats sacrificed immediately after gavage in the same experiment. Percent gastric contents remaining = (absorbance at 20 min)/(absorbance at 0 min) x 100.

In baseline studies, with no drug treatment, gastric emptying over 20 min was determined. In dose-response studies, rats were treated with 0, 0.01, 0.1, 0.3, 1, 5, 10, or 100 μ g of exendin 3, exendin 4, or GLP-1(7-36)NH₂. The results are shown in Figure 2. Figure 2 shows that exendins 3 and 4 inhibited gastric emptying with approximately the same ED₅₀ of 0.1 μ g, whereas GLP-1(7-36)NH₂ has an ED₅₀ of approximately 9 μ g, indicating that the exendins are ~90 fold more potent than GLP-1 at inhibiting gastric emptying.

As shown in Table I, exendin-3 and exendin-4 were found to be potent inhibitors of gastric emptying. The effect of rat amylin on gastric emptying is also provided as a second positive control and for comparative purposes.

TABLE I

DOSE μ g	GLP-1 (7-36)NH ₂		Exendin-3		Exendin-4		Rat Amylin	
	% remaining *(n)	SEM	% remaining *(n)	SEM	% remaining *(n)	SEM	% remaining *(n)	SEM
Saline Control	48.00 (16)	3.50	46.760 (15)	2.360	46.000 (17)	2.000	48.00 (17)	3.5
0.010	no data		58.240 (3)	3.180	no data	2.000	37.60 (2)	2.50
0.100	42.00 (7)	6.50	70.770 (3)	5.600	72.000 (3)	12.000	52.70 (6)	6.30
0.300	29.60 (7)	3.50	86.420 (3)	6.160	98.000 (2)	4.000	88.20 (4)	3.00
1.000	37.20 (9)	2.70	95.330 (3)	0.790	103.000 (1)	0.000	96.80 (9)	2.80
3.000	56.60 (10)	6.10					108.00(4)	2.70
10.000	87.90 (11)	2.70	101.760 (3)	3.390	112.000 (3)	2.000	101.10 (6)	3.60
100.000	103.60 (7)	2.80	103.640 (3)	2.260	103.000 (3)	3.000	101.20 (2)	2.80

*percent of gastric contents remaining 20 minutes after gavage.

EXAMPLE 2

The effects of exendin-4 analogs on inhibition of gastric emptying were examined, and compared to the effects of exendin-4, according to the methods described in Example 1. Male HSD rats were treated with 0.01, 0.1, 0.3, 1, 10 and 100 μ g of exendin-4, 0.01, 0.03, 0.1, 1, 10 and 100 μ g exendin-4 acid, and 0.1, 0.3, 1, 10 and 100 μ g of 14 Leu, 25 Phe exendin-4. Exendin-3, exendin-4 acid and 14 Leu, 25 Phe were synthesized according to standard peptide synthesis methods. The results, shown in Figure 3 and Table II, show that the exendin agonists, exendin-4 acid and 14 Leu, 25 Phe exendin-4, are potent inhibitors of gastric emptying. The EC_{50} of exendin-4 was 0.27 μ g. The EC_{50} s of exendin-4 acid and 14 Leu, 25 Phe exendin-4 were comparable (0.12 μ g and 0.29 μ g, respectively).

TABLE II

	Compound	EC_{50} (μ g)
	exendin-4	0.27
20	exendin-4 acid	0.12
	14 Leu, 25 Phe exendin-4	0.29

EXAMPLE 3

The ability of exendin[9-39], an antagonist of exendin's effects at the cloned GLP-1 receptor, to antagonize the gastric emptying inhibition effect of exendin-4 and GLP-1[7-36]NH₂, was examined according to the methods described in Example 1. Rats were treated with 1.0 μ g exendin-4, 1.0 μ g exendin-4 with 0.3 mg exendin[9-39], 10 μ g GLP-1[7-36]NH₂, 10 μ g GLP-1[7-36]NH₂ with 0.3 mg exendin[9-39] or with 0.3 mg exendin 9-39 alone. In these studies, exendin[9-39] was give both subcutaneously (sc)

and intravenously (iv). The results of these experiments are shown in Figures 4-7.

As shown in Figures 4 and 5, after 20 minutes, the animals treated with exendin-4 showed extremely potent inhibition of gastric emptying, which was not reversed by exendin[9-39]. This occurred regardless of whether the exendin[9-39] was administered sc or iv. Exendin[9-39] alone had no effect on gastric emptying.

As discussed above, exendin[9-39] is a potent antagonist of GLP-1 which binds at the cloned GLP-1 receptor (Fehmann HC, et al., *Peptides* 15(3): 453-6, 1994; Thorens B, et al., *Diabetes* 42(11): 1678-82, 1993). Surprisingly, however, exendin[9-39] did not block the effect of exendin-4 on gastric emptying (see Figures 4 and 5). These results indicate that the effects of exendins on gastric emptying are not due binding of the exendins at the cloned GLP-1 receptor, but instead that the gastric emptying effects of exendins are due to a different receptor.

That exendin[9-39] did not block the effect of GLP-1[7-36]NH₂ on gastric emptying (see Figures 6 and 7) indicates that, in its effects on gastric emptying, GLP-1 is also acting at a receptor other than the cloned GLP-1 receptor (at which exendin[9-39] is a potent antagonist).

25

EXAMPLE 4

Preparation of amidated peptide having SEQ. ID. NO. [5].

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.). In

general, single-coupling cycles were used throughout the synthesis and Fast Moc (HBTU activation) chemistry was employed. However, at some positions coupling was less efficient than expected and double couplings were required. In particular, residues Asp₆, Thr, and Phe₈ all required double coupling. Deprotection (Fmoc group removal) of the growing peptide chain using piperidine was not always efficient. Double deprotection was required at positions Arg₂₀, Val₁₉, and Leu₁₄. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized peptide was dissolved in water). Crude purity was about 55%.

Used in purification steps and analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

The solution containing peptide was applied to a preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions were pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.5 minutes. Electrospray Mass Spectrometry (M): calculated 4131.7; found 4129.3.

EXAMPLE 5Preparation of Peptide having SEQ. ID. NO. [6]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 25% to 75% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 21.5 minutes. Electrospray Mass Spectrometry (M): calculated 4168.6; found 4171.2.

15

EXAMPLE 6Preparation of Peptide having SEQ. ID. NO. [7]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 17.9 minutes. Electrospray Mass Spectrometry (M): calculated 4147.6; found 4150.2.

EXAMPLE 7Preparation of Peptide having SEQ. ID. NO. [8]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 35% to 65% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 19.7 minutes. Electrospray Mass Spectrometry (M): calculated 4212.6; found 4213.2.

15

EXAMPLE 8Preparation of Peptide having SEQ. ID. NO. [9]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 50% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 16.3 minutes. Electrospray Mass Spectrometry (M): calculated 4262.7; found 4262.4.

EXAMPLE 9Preparation of Peptide having SEQ. ID. NO. [10]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4172.6

15

EXAMPLE 10Preparation of Peptide having SEQ. ID. NO. [11]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in
25 Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4224.7.

EXAMPLE 11Preparation of Peptide having SEQ. ID. NO. [12]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4172.6

15

EXAMPLE 12Preparation of Peptide having SEQ. ID. NO. [13]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4186.6

EXAMPLE 13Preparation of Peptide having SEQ. ID. NO. [14]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4200.7

15

EXAMPLE 14Preparation of Peptide having SEQ. ID. NO. [15]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in
25 Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4200.7

EXAMPLE 15Preparation of Peptide having SEQ. ID. NO. [16]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4202.7.

15

EXAMPLE 16Preparation of Peptide having SEQ. ID. NO. [17]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in
25 Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4145.6.

EXAMPLE 17Preparation of Peptide having SEQ. ID. NO. [18]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4184.6.

15

EXAMPLE 18Preparation of Peptide having SEQ. ID. NO. [19]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in
25 Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4145.6.

EXAMPLE 19Preparation of Peptide having SEQ. ID. NO. [20]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4224.7.

15

EXAMPLE 20Preparation of Peptide having SEQ. ID. NO. [21]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in
25 Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4172.6.

EXAMPLE 21Preparation of Peptide having SEQ. ID. NO. [22]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4115.5.

EXAMPLE 22Preparation of Peptide having SEQ. ID. NO. [23]

15 The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 4. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in
25 Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4188.6.

EXAMPLE 23

Preparation of Peptide having SEQ. ID. NO. [24]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 1. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4131.6.

15

EXAMPLE 24

Preparation of Peptide having SEQ. ID. NO. [25]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 1. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in
25 Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4172.6.

EXAMPLE 25Preparation of Peptide having SEQ. ID. NO. [26]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 1. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in
Solvent A over 30 minutes) of the lyophilized peptide is
then carried out to determine the retention time of the
product peptide. Electrospray Mass Spectrometry (M):
calculated 4145.6.

15

EXAMPLE 26Preparation of Peptide having SEQ. ID. NO. [27]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 1. Additional double couplings
are required at the thioproline positions 38, 37, 36 and
31. Used in analysis are Solvent A (0.1% TFA in water)
25 and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC
(gradient 30% to 60% Solvent B in Solvent A over 30
minutes) of the lyophilized peptide is then carried out
to determine the retention time of the product peptide.
Electrospray Mass Spectrometry (M): calculated 4266.8.

EXAMPLE 27Preparation of Peptide having SEQ. ID. NO. [28]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 1. Additional double couplings
are required at the thioproline positions 38, 37 and 36.
10 Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC
(gradient 30% to 60% Solvent B in Solvent A over 30
minutes) of the lyophilized peptide is then carried out
to determine the retention time of the product peptide.
15 Electrospray Mass Spectrometry (M): calculated 4246.8.

EXAMPLE 28Preparation of Peptide having SEQ. ID. NO. [29]

The above-identified peptide is assembled on 4-(2'-
4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 1. Additional double couplings
are required at the homoproline positions 38, 37, 36 and
25 31. Used in analysis are Solvent A (0.1% TFA in water)
and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC
(gradient 30% to 60% Solvent B in Solvent A over 30
minutes) of the lyophilized peptide is then carried out
to determine the retention time of the product peptide.

Electrospray Mass Spectrometry (M): calculated 4250.8.

EXAMPLE 29

Preparation of Peptide having SEQ. ID. NO. [30]

The above-identified peptide is assembled on 4-(2'-
5 4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 1. Additional double couplings
10 are required at the homoproline positions 38, 37, and
36. Used in analysis are Solvent A (0.1% TFA in water)
and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC
(gradient 30% to 60% Solvent B in Solvent A over 30
minutes) of the lyophilized peptide is then carried out
15 to determine the retention time of the product peptide.
Electrospray Mass Spectrometry (M): calculated 4234.8.

EXAMPLE 30

Preparation of Peptide having SEQ. ID. NO. [31]

The above-identified peptide is assembled on 4-(2'-
20 4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 1. Additional double couplings
25 are required at the thioproline positions 38, 37, 36 and
31. Used in analysis are Solvent A (0.1% TFA in water)
and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC
(gradient 30% to 60% Solvent B in Solvent A over 30

minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4209.8.

EXAMPLE 31

5 Preparation of Peptide having SEQ. ID. NO. [32]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
10 cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the homoproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC
15 (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4193.7.

EXAMPLE 32

20 Preparation of Peptide having SEQ. ID. NO. [33]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
25 cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the N-methylalanine positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in

water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.

5 Electrospray Mass Spectrometry (M): calculated 3858.2.

EXAMPLE 33

Preparation of Peptide having SEQ. ID. NO. [34]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

10 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the N-methylalanine positions 38, 37 and

15 36. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.

20 Electrospray Mass Spectrometry (M): calculated 3940.3.

EXAMPLE 34

Preparation of Peptide having SEQ. ID. NO. [35]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

25 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings

are required at the N-methylalanine positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3801.1.

EXAMPLE 35

Preparation of C-terminal carboxylic acid Peptides
10 corresponding to the above C-terminal amide sequences.

The above peptides are assembled on the so called Wang resin (p-alkoxybenzylalcohol resin (Bachem, 0.54 mmole/g)) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry provides an experimentally determined (M).

WE CLAIM:

1. A method of beneficially regulating gastro-intestinal motility in a subject comprising administering to said subject a therapeutically effective amount of an exendin or an exendin agonist.

2. A method according to claim 1 wherein said beneficial regulation of gastrointestinal motility comprises reducing gastric motility.

3. A method according to claim 1 wherein said beneficial regulation of gastrointestinal motility comprises delaying gastric emptying.

4. The method according to claim 1, 2 or 3 wherein said exendin is exendin 3.

5. The method according to claim 1, 2 or 3 wherein said exendin agonist is exendin-4.

6. The method according to claim 1, 2 or 3 wherein said subject is undergoing a gastrointestinal diagnostic procedure.

7. The method of claim 6 wherein said gastrointestinal diagnostic procedure is a radiological examination.

8. The method of claim 7 wherein said gastrointestinal diagnostic procedure is magnetic resonance imaging.

9. A method according to claim 1, 2 or 3 wherein said gastric motility is associated with a gastrointestinal disorder.

10. A method according to claim 9 wherein said
5 gastrointestinal disorder is a spasm.

11. A method according to claim 10 wherein said spasm is associated with a disorder selected from the group consisting of acute diverticulitis or a disorder of the biliary tract or a disorder of the Sphincter of
10 Oddi.

12. A method of treating postprandial dumping syndrome in a subject comprising administering to said subject a therapeutic effective amount of an exendin or exendin agonist.

15 13. A method of treating postprandial hyperglycemia comprising administering a therapeutically effective amount of an exendin or exendin agonist.

14. The method according to claim 13 further comprising administering a therapeutically effective
20 amount of an amylin or an amylin agonist.

15. The method according to claim 14 wherein said amylin agonist is ²⁵Pro, ²⁶Pro, ²⁹Pro-h-amylin.

16. A method of treating postprandial
25 hyperglycemia which is a consequence of type 2 diabetes mellitus comprising administering a therapeutically

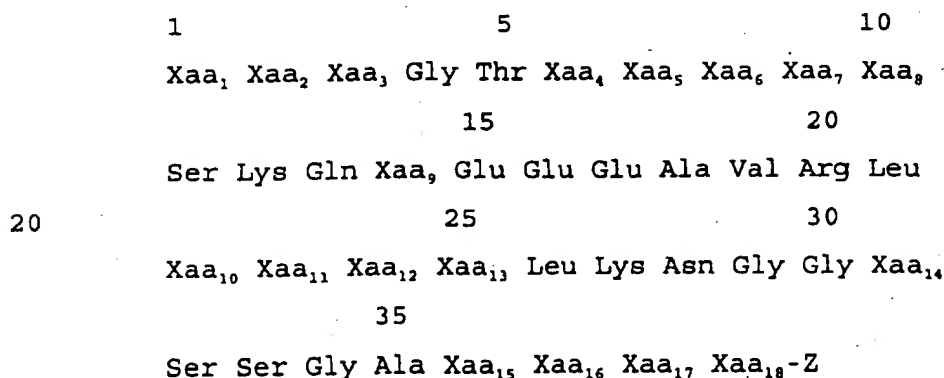
effective amount of an exendin or an exendin agonist.

17. A method of treating type 1 diabetes mellitus comprising administering a therapeutically effective amount of an exendin or an exendin agonist.

5 18. A method of treating impaired glucose tolerance comprising administering a therapeutically effective amount of an exendin or an exendin agonist.

19. A method of treatment for ingestion of a toxin comprising: (a) administering an amount of an exendin
10 or an exendin agonist effective to prevent or reduce the passage of stomach contents to the intestines; and (b) aspirating the contents of the stomach.

20. The method according to claim 1, 2 or 3 wherein said exendin agonist is selected from a peptide
15 compound of the formula:



wherein Xaa₁ is His, Arg or Tyr;
25 Xaa₂ is Ser, Gly, Ala or Thr;
Xaa₃ is Asp or Glu;

Xaa₁ is Phe, Tyr or naphthylalanine;
 Xaa₂ is Thr or Ser;
 Xaa₃ is Ser or Thr;
 Xaa₄ is Asp or Glu;
 5 Xaa₅ is Leu, Ile, Val, pentylglycine or Met;
 Xaa₆ is Leu, Ile, pentylglycine, Val or Met;
 Xaa₁₀ is Phe, Tyr or naphthylalanine;
 Xaa₁₁ is Ile, Val, Leu, pentylglycine,
 tert-butylglycine or Met;
 10 Xaa₁₂ is Glu or Asp;
 Xaa₁₃ is Trp, Phe, Tyr, or naphthylalanine;
 Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently
 Pro, homoproline, 3Hyp, 4Hyp,
 thioproline, N-alkylglycine,
 15 N-alkylpentylglycine or N-alkylalanine;
 Xaa₁₈ is Ser, Thr or Tyr; and
 Z is -OH or -NH₂;
 with the proviso that the compound does not
 have the formula of either exendin-3 [SEQ. ID.
 20 NO. 1] or exendin-4 [SEQ. ID. NO. 2] and
 pharmaceutically acceptable salts thereof.

21. The method according to claim 1, 2 or 3
 wherein said exendin agonist is selected from a peptide
 compound of the formula [SEQ. ID. NO. 36]:

25	1	5	10
	Xaa ₁	Xaa ₂	Xaa ₃
	Xaa ₄	Gly	Thr
	Xaa ₅	Xaa ₆	Xaa ₇
	Xaa ₈	Xaa ₉	Xaa ₁₀
	15	20	
	Ser	Lys	Gln
	Xaa ₁₁	Glu	Glu
	Xaa ₁₂	Glu	Ala
	25	30	
	Xaa ₁₃	Leu	Lys
30	Xaa ₁₄	Asn	Gly
	Xaa ₁₅	Gly	Xaa ₁₆
	35		
	Ser	Ser	Gly
	Ala	Xaa ₁₇	Xaa ₁₈ -Z

wherein Xaa₁ is His or Arg;
Xaa₂ is Ser, Gly;
Xaa₃ is Asp or Glu;
Xaa₄ is Phe or naphthylalanine;
5 Xaa₅ is Thr or Ser;
Xaa₆ is Ser or Thr;
Xaa₇ is Asp or Glu;
Xaa₈ is Leu or pentylglycine;
Xaa₉ is Leu or pentylglycine;
10 Xaa₁₀ is Phe or naphthylalanine;
Xaa₁₁ is Ile, Val or tert-butylglycine;
Xaa₁₂ is Glu or Asp;)
Xaa₁₃ is Trp or Phe;
Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently
15 Pro, homoproline, thioproline or
N-methylalanine;
Xaa₁₈ is Ser or Tyr; and
Z is -OH or -NH₂;
with the proviso that the compound does not
20 have the formula of either exendin-3 [SEQ. ID.
NO. 1] or exendin-4 [SEQ. ID. NO. 2] and
pharmaceutically acceptable salts thereof.

Figure 1

GLP-1 (7-37)
HAEGTFTSDV SSYLEGQAAK EFIAWLVKGR G-NH₂

Exendin-3
HSDGTFITDL SKQMEEEEAVR LFIEWLKNKG PSSGAPPPS-NH₂

Exendin-4
HGEFTFTIDL SKQMEEEEAVR LFIEWLKNKG PSSGAPPPS-NH₂

Exendin[9-39]
DL SKQMEEEEAVR LFIEWLKNKG PSSGAPPPS-NH₂

FIGURE 2

Gastric emptying with exendins

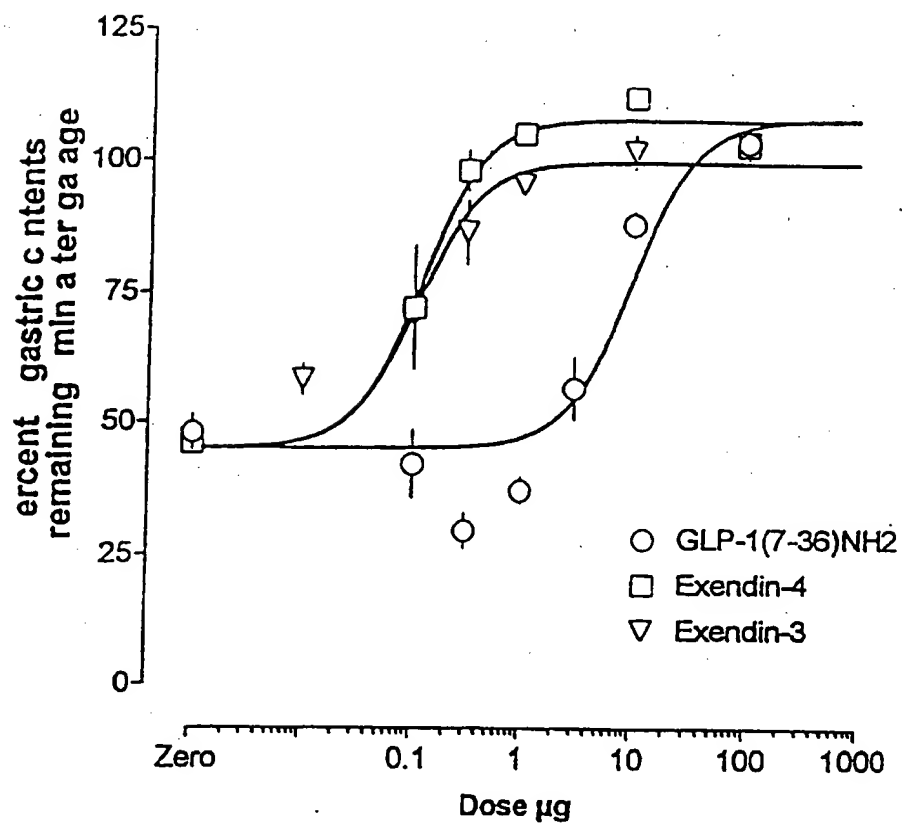


Figure 3

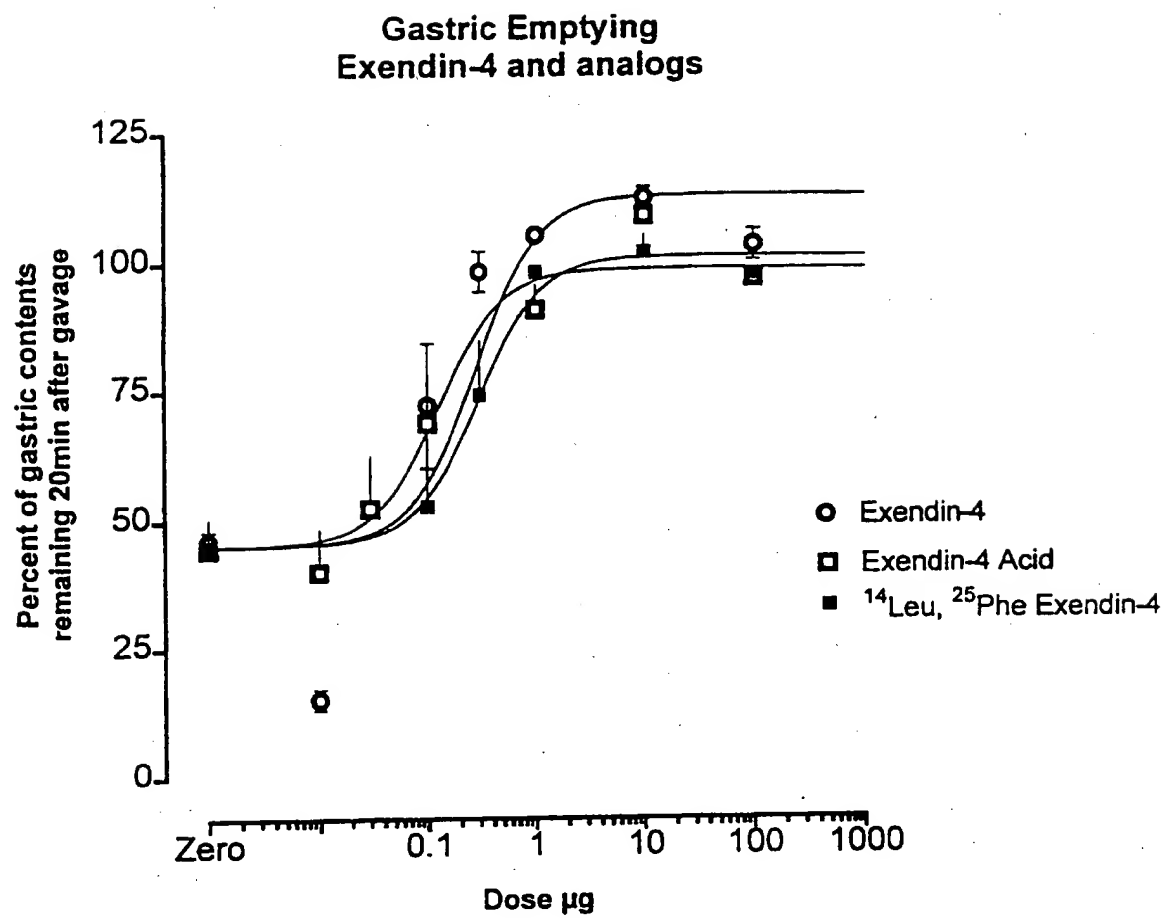


Figure 4

Exendin 9-39 given sc did not
antagonize the effect of Exendin-4 on
gastric emptying

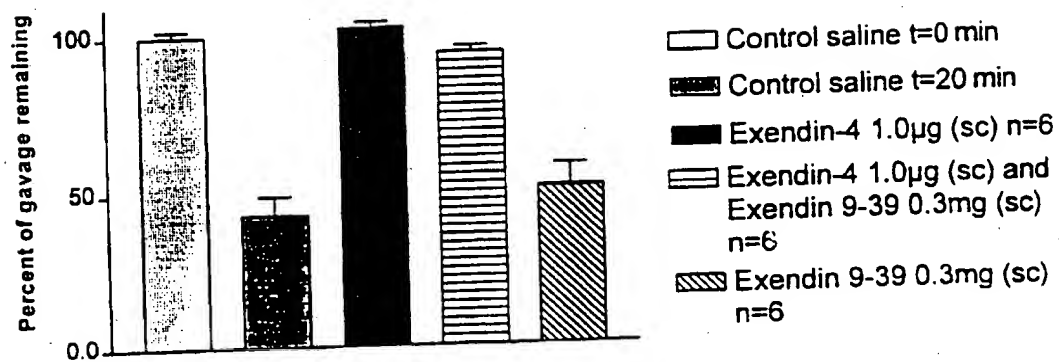


Figure 5

Exendin 9-39 given iv did not antagonize
the effect of Exendin-4 on gastric
emptying

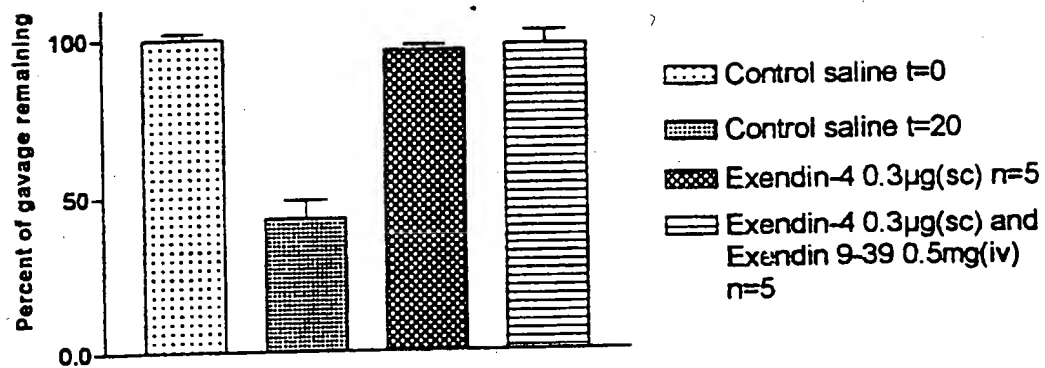


Figure 6

Exendin 9-39 given sc did not
antagonize the effect of GLP-1 on
gastric emptying

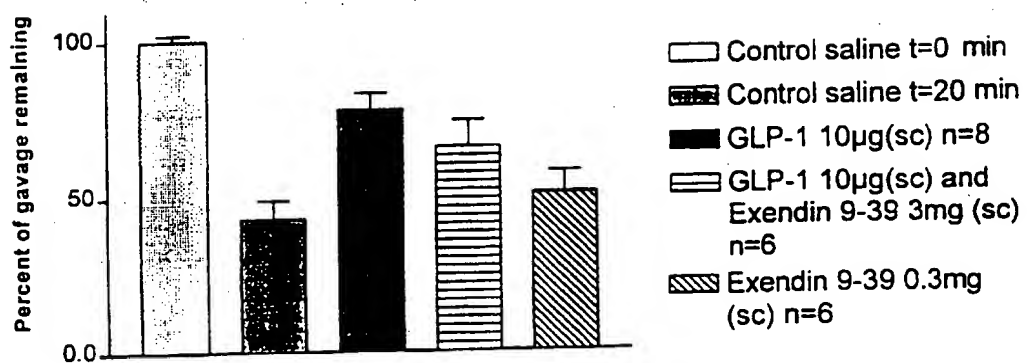


Figure 7

Exendin 9-39 given iv did not antagonize
the effect of GLP-1 on gastric emptying

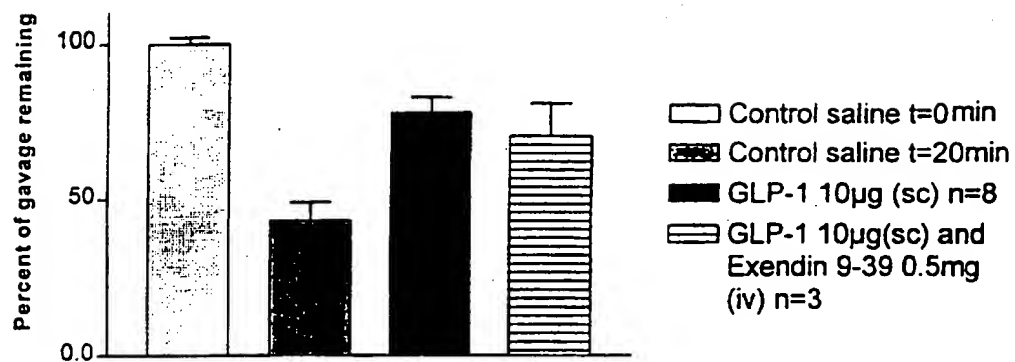


FIGURE 8-1

1 Xaa, Xaa, Xaa, Gly Thr Xaa, Xaa, Xaa, Xaa, Ser Lys Gln Xaa, Glu Glu Glu Ala Val Arg Leu
 5 10 15 20
 25 30 35
 Xaa₁₀ Xaa₁₁ Xaa₁₂ Xaa₁₃ Leu Lys Asn Gly Gly Xaa₁₄ Ser Ser Gly Ala Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈-Z

Compound (Seq. ID. No.)	Xaa ₁	Xaa ₂	Xaa ₃	Xaa ₄	Xaa ₅	Xaa ₆	Xaa ₇	Xaa ₈	Xaa ₉	Xaa ₁₀	Xaa ₁₁	Xaa ₁₂	Xaa ₁₃	Xaa ₁₄	Xaa ₁₅	Xaa ₁₆	Xaa ₁₇	Xaa ₁₈	2
1 [5]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Ser	NH ₂		
2 [6]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		
3 [7]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Ser	NH ₂		
4 [8]	Tyr	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		
5 [9]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Tyr	NH ₂		
6 [10]	His	Gly	Asp	Phe	Thr	Ser	Asp	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		
7 [11]	His	Gly	Glu	naph	Thr	Ser	Asp	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		
8 [12]	His	Gly	Glu	Phe	Ser	Ser	Asp	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		
9 [13]	His	Gly	Glu	Phe	Ser	Thr	Asp	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		
10 [14]	His	Gly	Glu	Phe	Thr	Thr	Asp	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		
11 [15]	His	Gly	Glu	Phe	Thr	Ser	Glu	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		
12 [16]	His	Gly	Glu	Phe	Thr	Ser	Asp	pGly	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		
13 [17]	His	Gly	Glu	Phe	Thr	Ser	Asp	pGly	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Ser	NH ₂		
14 [18]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Ser	NH ₂		

FIGURE 8-2

Compound (SEQ. ID. NO.)	Xaa ₁	Xaa ₂	Xaa ₃	Xaa ₄	Xaa ₅	Xaa ₆	Xaa ₇	Xaa ₈	Xaa ₉	Xaa ₁₀	Xaa ₁₁	Xaa ₁₂	Xaa ₁₃	Xaa ₁₄	Xaa ₁₅	Xaa ₁₆	Xaa ₁₇	Xaa ₁₈	Z
15 [19]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	pGly	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH ₂
16 [20]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	naph	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH ₂
17 [21]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Val	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH ₂
18 [22]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Val	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH ₂
19 [23]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	tBuG	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH ₂
20 [24]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	tBuG	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH ₂
21 [25]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Asp	Trp	Pro	Pro	Pro	Pro	Ser	NH ₂
22 [26]	His	Ala	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH ₂
23 [27]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	tPro	tPro	tPro	Ser	NH ₂
24 [28]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	tPro	tPro	tPro	Ser	NH ₂
25 [29]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	tPro	tPro	hPro	Ser	NH ₂
26 [30]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	tPro	tPro	hPro	Ser	NH ₂
27 [31]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	Pro	tPro	tPro	hPro	Ser	NH ₂
28 [32]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	Pro	tPro	tPro	hPro	Ser	NH ₂
29 [33]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	MeAla	MeAla	MeAla	MeAla	Ser	NH ₂
30 [34]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	MeAla	MeAla	MeAla	Ser	NH ₂
31 [35]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	MeAla	MeAla	MeAla	MeAla	Ser	NH ₂

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14199

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/00, 38/26; G03F 5/00; C07K 2/00, 5/00

US CL : 514/2, 12; 430/30; 530/300, 303, 308

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 12; 430/30; 530/300, 303, 308

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database on Derwent Information Ltd. AN 93-37955. HELLSTROM et al. GLIP (Glucagon-Like Insulinotropic Peptide)	1-3
Y	Delays Gastric Emptying as Part of its Antidiabetogenic Effect in Non-Insulin Dependent Diabetes Mellitus (NIDDM). Scand. J. Gastroenterol. 1993, Vol 28, Suppl. 197, page 38, see included Abstract.	1-11
Y	Database on Derwent Information Ltd. AN 95-39473. SCHIRRA et al. Differential effects of subcutaneous GLP-1 on gastric emptying, insulin release and exocrine pancreatic secretion in men. Gastroenterology. 1995, Vol. 108, No. 4, Suppl., A1003, see included Abstract.	1-4 and 6-11

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

25 SEPTEMBER 1997

Date of mailing of the international search report

24 OCT 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14199

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	SCHIRRA et al. Differential effects of subcutaneous GLP-1 on gastric emptying, antroduodenal motility, and pancreatic function in men. Proceedings of the Association of American Physicians. January, 1997, Vol 109, No. 1, pp. 84-97, Abstract.	1-3
Y	RAI et al. Actions of Helodermatidae venom peptides and mammalian glucagon-like peptides on gastric chief cells. Am. Physiol. J. 1993, Vol. 265 pages G118-G125, see especially Abstract and page G118.	1-5
Y	DANIEL et al. Use of Glucagon in the Treatment of Acute Diverticulitis. Br. Med. J. 21 September 1974, Vol. 3, pages 720-722, see especially Abstract and Page 20.	9-11
Y	US 3,862,301 A (CHERNISH et al.) 18 June 1973, see column 1.	1-3, 6-8
A	MIHOLIC et al. Glucagon-like peptide-1 (GLP-1), Entleerung Des Magnersaltzes und das Dumpingsyndrom nach Gastrektomie: Gastric substitute emptying, GLP-1, and dumping after total gastrectomy. Chirurgisches Forum. 1991, pages 429-232, see included Abstract.	12
X	NAUCK et al. Effects of subcutaneous Glucagon-like Peptide 1 (GLP-1[7-36 amide]) in Patients with Type 2-Diabetes. 1995. Diabetologia. Vol 38, Suppl. 1, page A39, Abstract No. 148, see entire Abstract	13 and 16
X	WO 95/07098 (AMYLIN PHARMACEUTICALS INC.) 16 March 1995, see especially pages 21 and 23.	14 and 15
X	DUPRE et al. Glucagon-Like Peptide I reduces Postprandial Glycemic Excursions in IDDM. Diabetes. June 1995, Vol 44, No. 6, pages 626-630, see Abstract.	17
X	D'ALESSIO et al. Glucagon-like Peptide I enhances Glucose Tolerance both by Stimulation of Insulin Release and by Increasing Insulin-Dependent Glucose Disposal. J. Clin. Invest. May 1994, Vol. 93, No. 5, pages 2263-2266, see Abstract.	18
A	BAYER et al. Advances in Poison Management. Clinical Chemistry. 1996, Vol. 42, No. 8(B), pages 1361-1366, see especially page 1365, last paragraph.	19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14199

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 20 and 21
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

No meaningful search could be carried out on claims 20 and 21 because sequences in computer readable form were not submitted with the application and further because claim 20 disclosed no SEQ ID NO. that could have been searched had sequences in computer readable form been submitted.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/14199

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, EMBASE, BIOSIS, CAPLUS, DRUGU, MEDLINE, SCISEARCH, TOXLINE, TOXLIT, JICST-EPLUS
search terms: postprandial hyperglycemia, glp-1, IDDM, NIDDM, treat, therapy, glucose tolerance, toxin, stomach
motility, gastric empty, poison, exendin

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I. Claims 1-11 and 20-21 are drawn to a method of regulating gastrointestinal motility.

Group II. Claim 12 is drawn to a method of treating postprandial dumping syndrome

Group III. Claims 13-16 are drawn to a method of treating post
prandial hyperglycemia.

Group IV. Claim 17 is drawn to a method of treating type 1 diabetes mellitus.

Group V. Claim 18 is drawn to a method of treating impaired
glucose tolerance.

Group VI. Claim 19 is drawn to a method of treatment for ingestion of a toxin.

The inventions listed as Groups do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the claims of inventions II-VI are drawn to materially distinct methods which differ at least in objectives, method steps, reagents and/or dosages and/or schedules used, response variables, and criteria for success and which do not relate to the special technical of regulating gastrointestinal motility recited in Group I.